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Identification of *Rattus tanezumi* and Y25F Mutations in the *Vkorc1* Gene of *Rattus* spp. in Orange County, California (Abstract)

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ABSTRACT: Roof rats (*Rattus rattus*) are common invasive pests in both urban and agricultural sites as well as a significant public health threat. The use of anticoagulant rodenticides to help control the rodent population poses a risk of developing resistance to these products. Several reports have associated the non-synonymous Single Nucleotide Polymorphism (nsSNP) Tyr25Phe (Y25F) of the vitamin K epoxide reductase subcomponent 1 (*Vkorc1*) gene to anticoagulant rodenticide resistance (Diaz and Kohn 2021). We conducted a nsSNP screen in the population of *Rattus* spp. in Orange County to determine the prevalence of the Y25F nsSNP in the sampled rodents. Thirty-seven live rat traps were set throughout the county and the species of each trapped rat was determined by sequencing the cytochrome oxidase I (COI) gene using primers previously described in Goulois et al. (2015). Twenty specimens were identified as *R. rattus* and 12 as *R. tanezumi* (Asian house rat); the other five specimens were non-*Rattus* species. To further evaluate the rat species, we used tree-based methods using sequence alignments for three mitochondrial DNA regions, cytochrome *b*, COI, and non-coding displacement (D) loop using a different set of primers used by Robins et al. (2007). Sequences were aligned by Geneious Prime (v.2020.2.4), and maximum-likelihood phylogeny using 1,000 bootstrap replications was constructed using Mega (10.2.6). Sequences were compared to reference sequences in Robins et al. (2007) and *Rattus norvegicus* (Norway rat) was used to root the tree. Phylogenetic analysis confirmed the presence of *R. tanezumi* in our collected samples as well as two haplotypes of *R. rattus*. In addition to the concatenated data set, each gene was examined individually, and all phylogenetic trees generally agreed on topology. The Y25F nsSNP was present in both *R. rattus* (40%; 8 of 20) and *R. tanezumi* (16.67%; 2 of 12). Further genetic testing in Orange County and other areas of Southern California are needed to determine the extent of these *Rattus* populations and their hybridization as well as the presence of this adaptive trait.

KEY WORDS: Asian house rat, phylogenetic analysis, *Rattus rattus*, *Rattus tanezumi*, roof rat, *Vkorc1* gene, Y25F mutation

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